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Use of sentinel chickens to evaluate the effectiveness of cleaning and disinfection procedures in noncommercial poultry operations infected with exotic Newcastle disease virus

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Abstract. The use of sentinel chickens in establishing the negative status of commercial poultry flocks depopulated due to exotic Newcastle disease (END) is considered to be an economically beneficial process. However, the costs and benefits of using sentinel chickens in noncommercial operations are in question. The objective of this study was to use sentinel chickens to evaluate whether adequate cleaning and disinfection coupled with an appropriate time period without susceptible poultry species on the premises would eliminate END virus from a noncommercial poultry operation and preclude the need for placement of sentinels in previously infected operations before declaring them free of virus. Noncommercial poultry operations were selected from the 2002 to 2003 END outbreak database. Operations included in the study had one or more isolations of END virus (ENDV) from cloacal or oropharyngeal swabs of birds on the premises. A total of 546 birds were placed on 53 premises. All sentinel birds sampled after placements were negative by virus detection methods and serologic tests. Results of this study indicate that time and the application of appropriate cleaning and disinfection procedures will adequately mitigate the risk of viable virus persisting in noncommercial poultry operations. In the future, this information may eliminate the need for sentinel bird placement to ensure virus free status of premises before repopulation, thereby decreasing the costs of END eradication.

Key words: Exotic Newcastle disease; sentinel chicken.

In October of 2002, exotic Newcastle disease (END) was confirmed by the National Veterinary Services Laboratories to have infected chickens in a noncommercial poultry operation in southern California. This was the index case of an outbreak that would subsequently affect 21 commercial and thousands of noncommercial poultry operations, aviaries, pet birds, and many other businesses and industries allied with poultry and nonpoultry concerns in California, Nevada, Arizona, and Texas.[5] Although discrete focal outbreaks of END had occurred in California before the 2002 to 2003 outbreak, no outbreak since the 1970s included commercial poultry operations. An outbreak of velogenic, viscertropic Newcastle disease in the 1970s had its index case in a noncommercial poultry flock from Fontana, California.[2] This outbreak ultimately infected or exposed 186 commercial operations and over 1,000 noncommercial operations and aviaries and required 3 years and the destruction of nearly 12 million birds to complete eradication of the virus from southern California.[2]

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Specific pathogen-free (SPF) sentinel chickens were used extensively in the 1970s outbreak. Because of extensive USDA-sponsored Newcastle disease virus (NDV) vaccination of commercial and noncommercial flocks during the outbreak, clinical signs in infected flocks were often unapparent. The placement of highly susceptible sentinel chickens allowed for rapid detection of virus if it was present. The sentinel bird program lasted 8 months, during which time approximately 37,000 sentinel chickens were placed on 458 commercial operations and in 2,594 noncommercial flocks.[2] These placements detected the presence of virus in 24 (5%) commercial and 3 (0.1%) noncommercial flocks.

The use of sentinel chickens is a common way to detect the presence of a potential pathogen. While not specifically addressing NDV or avian Paramyxovirus 1 (APMV-1), sentinel chickens have been used to determine the effectiveness of vaccination programs within an integrated broiler production program[8]; the potential pathogen challenge within a flock[9]; the presence of a pathogen and the timing of infection[7]; and the presence and transmission of a pathogen to other farms and flocks.[6] Public health agencies routinely use sentinel chickens in programs designed for early detection of arthropod-borne viruses including but not limited to Western and Eastern equine encephalomyelitis, Saint Louis encephalitis, and, most recently, West Nile virus.[4,10,11] The relative ease of placing and maintaining SPF sentinel chickens, their susceptibility to many pathogens, and the relative low cost make them an attractive monitoring and surveillance tool for poultry diseases as well as certain human diseases.

During the 2002 to 2003 END outbreak in southern California, an extensive vaccination campaign was not

initiated, the reasons for which are beyond the scope of this paper. Most commercial operations did incorporate NDV vaccination as part of their normal disease prevention protocols. The NDV vaccination status of most noncommercial operations was unknown, although very few were believed to have administered the vaccine. While use of sentinel chickens in commercial poultry operations that had been depopulated due to END was considered an important process in establishing the negative status of these flocks, the cost–benefit ratio of using sentinels in noncommercial operations was questioned. Newcastle disease virus is a relatively stable virus in the environment, but is susceptible to heat, irradiation, oxidation, pH effects, and chemical treatment.[1] Application of any one of these measures alone does not guarantee that the virus will be eliminated. However, these measures reduce the probability that an infectious dose will be present. The objective of this study was to use sentinel birds to conduct environmental monitoring and to evaluate whether adequate cleaning and disinfection coupled with an appropriate time period in which the operation remained empty of susceptible poultry species would eliminate END virus from a noncommercial poultry operation precluding the need for placement of sentinels in all previously infected operations before declaring them free of virus.

Noncommercial poultry operations were selected from the 2002 to 2003 END outbreak database. Operations for inclusion in the study had one or more isolations of ENDV from cloacal or oropharyngeal swabs of birds on the premises. Selected premises were either classified in the database as infected or dangerous contact premises and had housed game fowl. Cleaning and disinfection of premises were performed according to the standard operating procedures of the END task force. Briefly, these included: a) the removal of trash and debris that could not be adequately cleaned and disinfected; b) pressure washing surfaces that poultry potentially contacted; and c) spraying 1% Virkon® S[a] (a balanced, stabilized blend of peroxygen compounds, surfactant, organic acids, and an inorganic buffer system) on all surfaces that poultry potentially contacted. Owners of selected premises agreed to wait at least 90 days before repopulating the premises. Study premises were given individual premises identification numbers.

Preplacement surveys were conducted before sentinel bird placement to determine the owner's willingness to participate and care for the sentinel birds and to visually evaluate the thoroughness of premises cleaning and disinfection. Preplacement survey premises diagrams were constructed and included dimensions of all buildings (house, garage, sheds, etc.), locations of trees for roosting, and the availability of cages on the premises. In addition, potential holding areas for sentinel birds were assessed for containment security.

Sentinel birds were 7-week-old SPF chickens[b] not vaccinated for NDV. All sentinel birds received wing bands for identification before placement. Prior to the sentinel birds' arrival to the premise, all feeders and waterers were cleaned and disinfected using a 1% solution of Virkon S and clean wood shavings put down for bedding. All sentinel birds were delivered to sentinel premises by END task force personnel who wore protective clothing and had never visited the infected premises. Preplacement premises diagrams were used to determine the number of sentinel birds required for each premises. Sentinel birds were placed approximately 10 to 30 days after cleaning and disinfection in a biosecurity area (an area limiting access of free-roaming chickens to sentinel chickens). Open premises with free-roaming chickens received a minimum of five sentinel birds. Any premises with chicken housing units received a minimum of three sentinel birds per unit. If sentinel birds were capable of being contained within their housing unit, they were allowed out of the cages; if they could not be contained within the housing unit, they were moved from cage to cage daily. Every attempt was made to expose sentinel chickens to all areas of the premises in which infected birds had either been housed or had access before depopulation. All equipment used to handle sentinel birds was cleaned and disinfected using a 1% solution of Virkon S. Premises owners conducted feeding, watering, daily observations of health status, and movement of sentinel birds around the premises. Sick or dead birds were promptly removed from the premises by task force personnel and submitted for diagnostic testing.

Sentinel birds were revisited 10 to 14 days after initial placement for observation. Observations recorded included the number of sentinel birds present, number of sick sentinel birds, and number of dead sentinel birds. All sentinel birds were examined and sampled 21 days after placement. Cloacal and oropharyngeal swabs and blood samples were collected from each sentinel bird. Cloacal swabs were placed in brain heart infusion (BHI) broth and stored in a cooler for transport. Blood samples were collected from wing veins into sterile blood collection tubes without anticoagulant.

Standard procedures for virus isolation were performed as follows: each of three, 9 to 11-day-old embryonated SPF chicken eggs was inoculated in the chorioallantoic sac with 0.2 ml of swab fluid mixed in a ratio of approximately 1.5 : 1 swab fluid to antibiotic mixture to suppress bacterial and fungal growth (penicillin, streptomycin, kanamycin, gentomycin, and mycostatin). For swab fluid with residual bacterial or fungal contamination following the first egg inoculation, the remaining swab fluid was passed through a 0.45-μm syringe filter before re-inoculation into eggs. Inoculated eggs were incubated at 37°C for either 3 or 5 days, and candled daily. For those eggs incubated 3 days, all amnioallantoic fluids, regardless of embryo mortality, were tested for the presence of hemagglutinating antigen (HA). For eggs incubated 5 days, amnioallantoic fluids were harvested and tested for HA activity only from eggs with embryo mortality. Amnioallantoic fluid from eggs with embryo mortality that did not agglutinate chicken erythrocytes were re-inoculated and if negative for embryo mortality on second passage, were identified as negative for APMV-1. Swab samples that were embryo lethal were tested for bacterial contaminants and the presence of common avian viral pathogens, such as avian influenza virus, avian infectious bronchitis virus, and infectious laryngotracheitis virus. Amnioallantoic fluids that demonstrated HA activity were characterized as APMV-1 positive

Table 1. Summary of premises-level variables for 53 premises previously confirmed with poultry infected with exotic Newcastle disease where sentinel chickens were placed.

Variable	Mean	Standard deviation	Range
Days from euthanasia and disposal to cleaning and disinfection	37.9	27.7	0–104
Days from euthanasia and disposal to sentinel bird placement	63.5	27.5	11–122
Days from cleaning and disinfection to sentinel bird placement	24.5	8.7	9–45
Number of sentinel birds placed per premises	10.1	5.0	2–25
Number of sentinel birds that died during study period	1.2	3.1	0–18

or negative by hemagglutination inhibition (HI) using APMV-1 antisera.

Following the virus detection and identification protocol used during END outbreak investigations, all original swab samples were also tested by real-time, reverse transcriptase-polymerase chain reaction (RRT-PCR) for ENDV and APMV-1 (lentogenic) NDV.[5] For any RRT-PCR positive samples, the PCR product would be sequenced and the pattern of amino acids at the fusion protein cleavage site used to confirm and differentiate lentogenic APMV-1 NDV, pigeon paramyxovirus-1 (PPMV-1) and ENDV.[5] Diagnostic sensitivity and specificity of the RRT-PCR assays used are reported as 0.9967 and 0.9999, respectively.[5] Serum samples were tested for antibodies to NDV using a commercially available ELISA[c].

Descriptive statistics (means and standard deviations) were calculated for premises-level variables including: days from euthanasia and disposal to cleaning and disinfection; days from euthanasia and disposal to placement of sentinel birds; days from cleaning and disinfection to placement of sentinel birds; number of sentinel birds placed; number of sentinel birds that became sick; and number of sentinel birds that died (Table 1). The total sentinel bird days was calculated by summing the total number of days all sentinel birds placed were under study across all sentinel premises.

A total of 546 sentinel birds were placed on 53 premises. All sentinel birds sampled were negative for ENDV by virus detection methods and to serologic tests. There were no sick birds identified at the recheck visits. There were, however, 15 premises (28%) that had 1 or more sentinel birds die during the study period. All birds dying before the end of the study were negative for ENDV by virus isolation and RRT-PCR on cloacal and oropharyngeal swabs. Specific causes of death other than predation were not determined. The total sentinel bird days were 10,962.

Placement of sentinel birds on noncommercial operations during the END outbreak in the1970s detected virus on 0.1% of the operations where they were placed. The cost of using sentinel birds must consider the initial cost of each bird, equipment, feed, housing, labor, and laboratory costs. The estimated cost of placement of each bird during the 1970s outbreak was $29.[2] A total of 13,281 birds were placed on noncommercial operations for a total cost of $385,149 or $128,383 per infected noncommercial operation detected by use of sentinel birds.

If it is assumed that, similar to the outbreak in the 1970s, a 0.1% flock prevalence following cleaning and disinfection was expected during the 2002 to 2003 outbreak, then 1,354 premises would have required sentinel bird placement for 95% confidence of detecting one or more positive flocks. The estimated cost of placement of each sentinel bird for this study was $58 (2003 US$). Therefore, if the 1,354 depopulated premises required, on average, the placement of 10 sentinel birds, the total cost would be approximately $785,000. These are substantial costs with very little benefit to disease control.

During the 2002 to 2003 END outbreak, a virus survival study was conducted on two infected commercial poultry operations where clinically affected chickens were confirmed to be infected with ENDV.[9] Environmental swab samples were collected daily for 21 days following depopulation, processed, and virus isolation procedures conducted. ENDV was never isolated from manure samples from one operation and was not isolated after day 16 following depopulation from the second operation. These operations were large layer ranches with many clinically affected birds. Virus shedding would be substantial with manure, the primary reservoir of shed virus. It was concluded that the dry and warm conditions in southern California resulted in rapid elimination of virus from the environments of these operations.

Results of this study indicate that time and the application of appropriate cleaning and disinfection procedures will adequately mitigate the risk of viable virus persisting in noncommercial poultry operations. In the future, this information may influence control measures used in END outbreaks by eliminating the need for placement of sentinel chickens to ensure the absence of virus. Precluding sentinel bird placement will decrease costs of END eradication through elimination of sentinel bird and equipment purchases as well as reduced labor costs.

Sources and manufacturers

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b. Charles River, North Franklin, CT, USA.
c. FlockCheck, IDEXX Laboratories, Westbrook, ME, USA.

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